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Comparative genomic analysis of *Clostridium perfringens* isolates from healthy and diseased livestock production animals

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Introduction

Clostridium perfringens type A is known to cause necrotic enteritis (NE) in poultry but may also cause necrotic enteritis and diarrhea in piglets. Virulence genes such as *netB*, that primarily are located on large toxin-encoding plasmids, seem to play a major role in the pathogenesis of NE in broiler chickens, whereas the significance of *netB* in turkeys is less well elucidated. *NetB* is found on a 42 kb plasmid-encoded pathogenicity island called NELoc-1 that harbors several other virulence genes. Properties of strains from diseased pigs are also sparsely known, however *cpb2* has been associated with NE in piglets. The aim of this study was to carry out comparative genomic analysis of *C. perfringens* isolates from healthy and diseased poultry and diseased pigs.

Material and Methods

Isolates from healthy (n=4) and diseased (n=6) chickens, healthy (n=4) and diseased (n=5) turkeys, and diseased pigs (n=5) were sequenced by paired-end Illumina sequencing. *De novo* assembled contigs were used for BLASTN analyses against previously published virulence factors and resistance genes. The relationship between the isolates was inferred by investigation of the genetic diversity in the core chromosome of the isolates against *C. perfringens* strain ATCC-13124, using maximum parsimony analysis.

Results

The genomes were sequenced to an average depth of >40X, with a total of 107,249 variant positions identified in ~60% of the reference strain, and conserved among all isolates. The phylogenetic analysis showed that isolates from broilers and turkeys were very diverse, whereas the pig isolates clustered more closely. Even the single type C isolate clustered together with the type A isolates from pigs. Four isolates from healthy poultry clustered together and did not carry the *netB* gene. The two pathogenicity islands NELoc-1 and -3 previously found to be associated to diseased poultry, were only found in NE isolates from broilers and a single NE isolate from a turkey, while *cpb2* was carried by all pig isolates and only a single NE isolate from a turkey. Many virulence genes were identified in the majority of all the isolates, and 23 isolates were of type A, and a single isolate of type C. Two of the isolates from healthy turkeys carried a gene with ~89 % sequence similarity to the *iap* iota toxin gene. The significance of this is unknown. Four different tetracycline resistance genes were identified, and 10 isolates carried tetracycline resistance genes. One chicken isolate carried the *ermB* gene, which confers resistance to

macrolides, while three different aminoglycoside resistance genes (*ant(6)-Ia*, *ant(6)-Ib* and *aph(3')-III*) were detected in three pig isolates.

Conclusions

NELoc-1 and -3 was present only in isolates from poultry suffering from necrotic enteritis, confirming the importance of *netB*

All isolates from diseased chickens were carrying NELoc-1 and -3 compared to only a single one from a turkey

cpb2 was detected in all the isolates from diseased pigs

The isolates from pigs were genetically more closely related to each other as compared to the isolates from poultry

Tetracycline resistance genes were common in all isolates regardless of host